

***Pestalotiopsis sonneratae* sp. nov. from China**

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ABSTRACT—A new pestalotioid species, *Pestalotiopsis sonneratae*, was isolated from leaf spot symptoms of *Sonneratia apetala* (mangrove apple) in Guangdong Province, China. The pathogen was characterized by morphological and molecular data ITS, *tef1*, and *tub2* loci. The species is described, illustrated, and compared with similar *Pestalotiopsis* species and another pestalotioid pathogen of *Sonneratia*.

KEY WORDS—*Amphisphaeriales*, phylogeny, *Sporocadaceae*, taxonomy

Introduction

Pestalotioid fungi are easily characterized by owning multi-septate and more or less fusiform conidia with appendages at one or both ends, frequently with some melanised cells (Liu & al. 2019). *Pestalotiopsis* Steyaert (*Sporocadaceae*, *Amphisphaeriales*) is a typical pestalotioid genus with 5-celled conidia, separated from *Pestalotia* with 6-celled conidia (Steyaert 1949). With molecular evidence, Maharachchikumbura & al. (2014) split *Pestalotiopsis* s.lat. into *Neopestalotiopsis*, *Pseudopestalotiopsis*, and *Pestalotiopsis* s.str. Subsequently, several new species were proposed based on morphology and phylogeny of combined ITS, *tef1*, and *tub2* loci (Gu & al. 2021, Liu & al. 2017, Norphanphoun & al. 2019).

Pestalotiopsis s.lat. is widely distributed in tropical and temperate regions, usually inhabiting plants as endophytes, pathogens and saprophytes (Ismail

& al. 2013, Maharachchikumbura & al. 2014). Several species are associated with economic plant diseases, for example, *Neopestalotiopsis rosicola* C.M. Tian & N. Jiang causes stem canker of *Rosa chinensis* (Jiang & al. 2018); *N. vitis* is the causal agent of *Vitis vinifera* (grapevine) leaf spot (Jayawardena & al. 2016); *Pestalotiopsis kenyana* Maharachch. & al. results in *Castanea mollissima* (Chinese chestnut) leaf disease (Jiang & al. 2021); several *Pestalotiopsis* species cause *Camellia oleifera* leaf blight (Li & al. 2021).

Sonneratia apetala Buch.-Ham. (Lythraceae; mangrove apple) is a main species of mangrove forests in southern China (Ren & al. 2009). Frequent brown leaf spots were discovered during our investigation of *S. apetala* diseases in Guangdong Province in China (FIG. 1). Here we propose the fungal pathogen as a new species, *Pestalotiopsis sonneratae*, based on modern taxonomic methods of morphology and phylogeny.



FIG. 1. Leaf spots on *Sonneratia apetala*.

Materials & methods

Samples and isolates

In this study, diseased mangrove apple leaves were collected, packed in paper bags and transferred to the laboratory for fungal isolation. The symptomatic leaves were primarily surface-sterilized for 1 min in 75% ethanol, 3 min in 1.25% sodium hypochlorite, and 1 min in 75% ethanol, then rinsed for 2 min in distilled water and blotted on dry sterile filter paper. Then the diseased areas of the leaves were cut into 0.5×0.5 cm pieces using an aseptic razor blade, and transferred onto the surface of potato dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per L) and malt extract agar (MEA; 30 g malt extract, 5 g mycological peptone, 15 g agar per L) plates, and incubated at 25°C to obtain pure cultures. The cultures were deposited in the China Forestry Culture Collection Center, Ecology and Nature Conservation Institute,

Chinese Academy of Forestry, Beijing, China (CFCC; <http://www.cfcc-caf.org.cn/>), and the specimens in the herbarium of the Chinese Academy of Forestry, Beijing, China (CAF; <http://museum.caf.ac.cn/>).

Morphological studies

Species identification was based on conidial morphology formed on PDA plates. Fifty conidia were selected randomly for measurement using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with a Nikon digital sight DS-Ri2 camera. Cultural characteristics were observed on PDA and MEA after 10 days incubation at 25°C in the dark.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fungal mycelium growing on MEA using a CTAB method (Doyle & Doyle 1990). The internal transcribed spacer of rDNA (ITS) was amplified using primers ITS1 and ITS4 (White & al. 1990). The translation elongation factor-1 alpha (*tef1*) was amplified using primers EF1-728F and EF2 (Carbone & Kohn 1999). The β -tubulin gene (*tub2*) was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). These regions were amplified as follows: an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 50 s at 52°C (ITS) or 54°C (*tef1* and *tub2*), and 1 min at 72°C, and a final elongation step of 7 min at 72°C. The PCR products were estimated visually by electrophoresis in 2% agarose gel set to 60 V for 90 min. DNA was sequenced using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v. 3.1 at the Shanghai Invitrogen Biological Technology Company Limited.

DNA sequence analysis

The new sequences generated in this study and the reference sequences of *Pestalotiopsis* species included in the phylogenetic analyses are summarized in TABLE 1. *Neopestalotiopsis magna* (MFLUCC 12-0652) was used as the outgroup taxon. These sequences were aligned with MAFFT v. 7 (Katoh & Standley 2013) and manually adjusted. Phylogenetic analyses were generated from the combined ITS-*tef1*-*tub2* sequences using PhyML v. 3.0 (Guindon & al. 2010) for maximum Likelihood (ML), and MrBayes v.3.1.2 for Bayesian Inference (BI) (Ronquist & Huelsenbeck 2003).

Results

Molecular phylogeny

The combined ITS, *tef1*, and *tub2* alignment comprised 45 sequences (including one outgroup taxon) and 1479 characters including alignment gaps, of which 1102 were parsimony informative, 240 were variable and parsimony-uninformative, and 137 were constant. The topologies resulting from ML and BI analyses of the concatenated dataset were congruent (FIG. 2). The new species appeared in a single clade with high bootstrap support (FIG. 2).

TABLE 1. Sequences of *Neopestalotiopsis* and *Pestalotiopsis* used in the molecular analysis.

SPECIES	ISOLATE	GENBANK ACCESSIONS NUMBERS		
		ITS	tub2	tef1
<i>N. magna</i>	MFLUCC 12-0652*	KF582795	KF582793	KF582791
<i>P. abietis</i>	CFCC 53011*	MK397013	MK622280	MK622277
	CFCC 53012	MK397014	MK622281	MK622278
<i>P. australasiae</i>	CBS 114126*	KM199297	KM199409	KM199499
	CBS 114141	KM199298	KM199410	KM199501
<i>P. biciliata</i>	CBS 124463*	KM199308	KM199399	KM199505
	CBS 236.38	KM199309	KM199401	KM199506
<i>P. brachiata</i>	LC2988*	KX894933	KX895265	KX895150
	LC8188	KY464142	KY464162	KY464152
	LC8189	KY464143	KY464163	KY464153
<i>P. camelliae-oleiferae</i>	CSUFTCC08*	OK493593	OK562368	OK507963
	CSUFTCC09	OK493594	OK562369	OK507964
<i>P. disseminata</i>	CBS 143904	MH554152	MH554825	MH554587
	MEAN 1165	MT374687	MT374712	MT374699
<i>P. dracontomelonis</i>	MFLU 14-0207*	KP781877	—	KP781880
<i>P. ericacearum</i>	IFRDCC 2439*	KC537807	KC537821	KC537814
<i>P. etonensis</i>	BRIO 66615*	NR_145237	KM199405	KM199509
<i>P. formosana</i>	NTUCC 17-009*	MH809381	MH809385	MH809389
<i>P. grevilleae</i>	CBS 114127*	KM199300	KM199407	KM199504
<i>P. kenyana</i>	CBS 442.67*	KM199302	KM199395	KM199502
<i>P. knightiae</i>	CBS 111963	KM199311	KM199406	KM199495
	CBS 114138*	KM199310	KM199408	KM199497
<i>P. macadamiae</i>	BRIP 63738b	KX186588	KX186680	KX186621
	BRIP 63739b	KX186587	KX186679	KX186620
<i>P. nanjingensis</i>	CSUFTCC16*	OK493602	OK562377	OK507972

SPECIES	ISOLATE	GENBANK ACCESSIONS NUMBERS		
		ITS	tub2	tef1
<i>P. nanningensis</i>	CSUFTCC10*	OK493596	OK562371	OK507966
<i>P. oryzae</i>	CBS 171.26	KM199304	KM199397	KM199494
	CBS 353.69*	KM199299	KM199398	KM199496
<i>P. parva</i>	CBS 265.37	KM199312	KM199404	KM199508
	CBS 278.35*	KM199313	KM199405	KM199509
<i>P. photiniicola</i>	GZCC 16-0028*	KY092404	KY047663	KY047662
<i>P. rhizophorae</i>	MFLUCC 17-0416*	MK764283	MK764349	MK764327
<i>P. rhodomyrti</i>	HGUP4230*	KF412648	KF412642	KF412645
<i>P. sonneratae</i>	CFCC 57392	ON114182	ON086814	ON086810
	CFCC 57393	ON114183	ON086815	ON086811
	CFCC 57394*	ON114184	ON086816	ON086812
	CFCC 57395	ON114185	ON086817	ON086813
<i>P. telopeae</i>	CBS 114137	KM199301	KM199469	KM199559
	CBS 114161*	KM199296	KM199403	KM199500
	CBS 113606	KM199295	KM199402	KM199498
<i>P. terricola</i>	CBS 141.69*	MH554004	MH554680	MH554438
<i>P. thailandica</i>	MFLUCC 17-1616*	MK764285	MK764351	MK764329
<i>P. trachycarpicola</i>	OP068*	JQ845947	JQ845945	JQ845946
	IFRDCC 2403	KC537809	KC537823	KC537816
	LC4523	KX895011	KX895344	KX895230

Ex-type strains are marked with *.

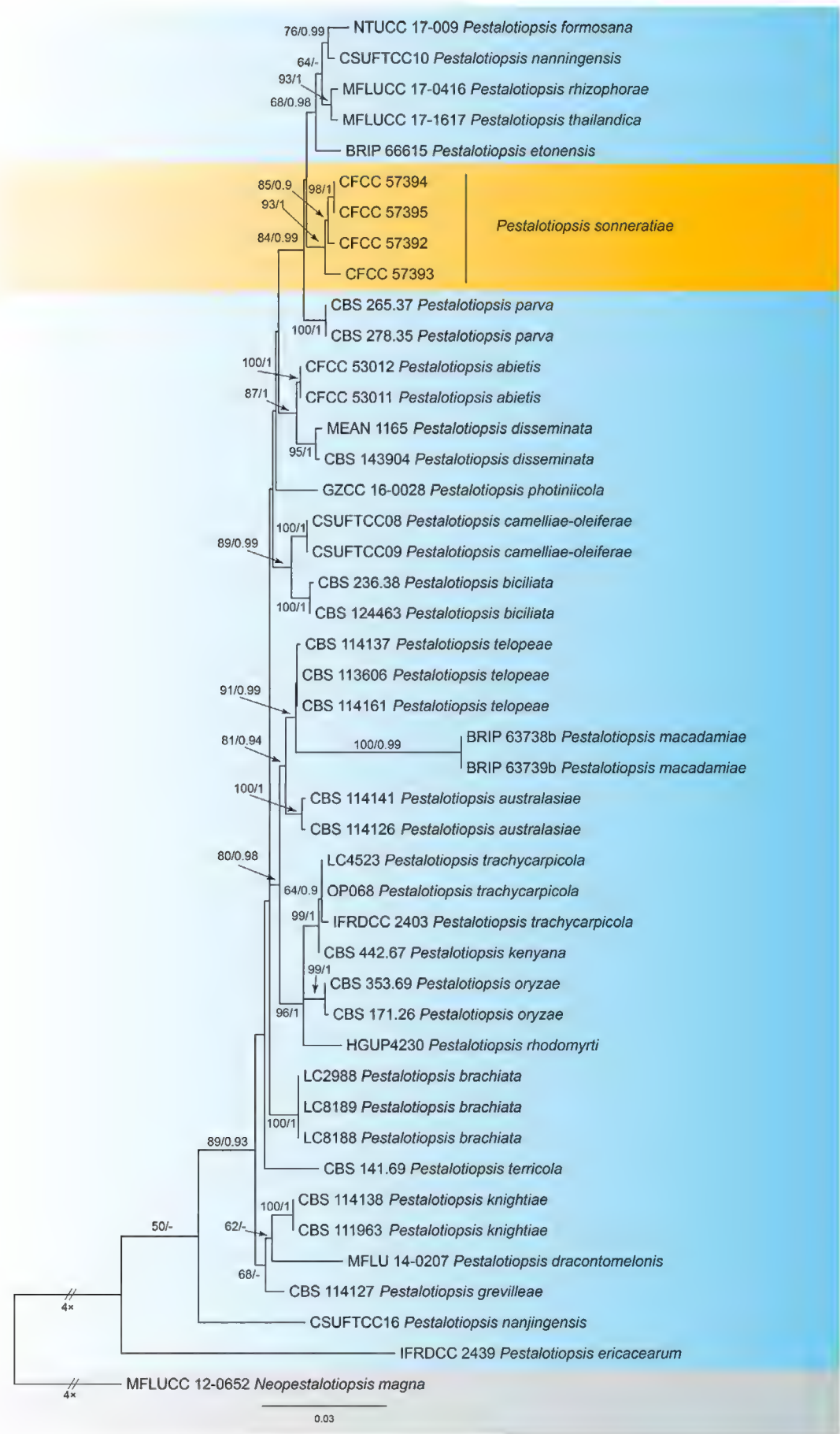


FIG. 2. Phylogram of *Pestalotiopsis* resulting from a maximum likelihood analysis based on a combined matrix of ITS, tef1, and tub2 loci. Numbers above the branches indicate ML bootstrap values (left, ML BS $\geq 50\%$) and Bayesian posterior probabilities (right, BPP ≥ 0.9). The tree is rooted with *Neopestalotiopsis magna* (MFLUCC 12-0652). Isolates from the present study are marked in blue.

Taxonomy

***Pestalotiopsis sonneratae* Ning Jiang, sp. nov.**

FIG. 3

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Differs from phylogenetically close *Pestalotiopsis etonensis*, *P. formosana*, *P. nanningensis*, *P. rhizophorae*, and *P. thailandica* by its host *Sonneratia* (Lythraceae, Malpighiales) and by sizable differences in its ITS, *tef1*, and *tub2* sequences.

TYPE: China, Guangdong Province, Zhongshan City, Hengmen village, 22.8056°N 113.9694°E, on leaves of *Sonneratia apetala*, 26 September 2018, coll. L.Y. Tian JNH0045 (**Holotype**, CAF800049; ex-type culture CFCC 57394; GenBank ON114184, ON086816, ON086812).

ETYMOLOGY: *sonneratae*, named after the host genus, *Sonneratia*.



FIG. 3. *Pestalotiopsis sonneratae* (ex-holotype, CFCC 57394). A. Colony on PDA after 10 d at 25°C; B. Colony on MEA after 10 d at 25°C; C. Conidioma formed on PDA; D, E. conidiogenous cells giving rise to conidia; F, G. conidia. Scale bars: C = 300 µm; D–G = 10 µm.

SEXUAL MORPH: Undetermined. ASEXUAL MORPH: CONIDIOMATA acervular, aggregated or solitary, erumpent, pulvinate, black, 100–350 µm diam., exuding black conidial masses. CONIDIOPHORES indistinct, usually reduced to conidiogenous cells. CONIDIOGENOUS CELLS hyaline, smooth, cylindrical to ampulliform, annelidic, $7.5\text{--}36.5 \times 1.5\text{--}5$ µm, means $\pm$ SD = $16 \pm 1.9 \times 3.5 \pm 0.7$ µm. CONIDIA fusoid, straight or slightly curved, 4-septate, smooth, slightly constricted at the septa, $(16\text{--})17.5\text{--}20.5(-22) \times (5.5\text{--})6\text{--}8(-8.5)$ µm, means $\pm$ SD = $19.1 \pm 1.4 \times 6.5 \pm 1.6$ µm (n = 50), L/W = 2.5–3.5; basal cell obconic with a truncate base, thin-walled, hyaline or pale brown, $(3\text{--})3.5\text{--}5(-5.5)$ µm; median cells 3, trapezoid or subcylindrical, concolourous, brown, thick-walled, the first median cell from base $(3\text{--})3.5\text{--}5$ µm long, the second cell $(3\text{--})3.5\text{--}4(-4.5)$ µm long, the third cell $(3\text{--})3.5\text{--}4.5(-5)$ µm long, together $(10.5\text{--})11\text{--}13(-14)$ µm long; apical cell conic with an acute apex, thin-walled, hyaline, $(2\text{--})2.5\text{--}3.5(-4)$ µm long; basal appendage single, unbranched, tubular, centric, straight or slightly bent, $2.5\text{--}5(-8)$ µm long, mean $\pm$ SD = 3.8 ± 1.3 µm; apical appendages 2–3, unbranched, tubular, centric, straight or bent, $(8\text{--})10\text{--}15.5(-18)$ µm long, mean $\pm$ SD = 12.7 ± 2.4 µm. COLONIES on MEA flat, with undulate edge, white to sienna, reaching 40 mm diam after 10 d at 25°C; on PDA flat, spreading, with flocculent aerial mycelium and entire edge, white, reaching 90 mm diam after 10 d at 25°C, forming black conidiomata with black conidial masses.

ADDITIONAL SPECIMEN EXAMINED: **CHINA, GUANGDONG PROVINCE, Zhongshan City**, Hengmen village, 22.8056°N 113.9694°E, on leaves of *Sonneratia apetala*, 8 June 2019, coll. L.Y. Tian (CFCC 57395; GenBank ON114185, ON086817, ON086813); (CFCC 57392; GenBank ON114182, ON086814, ON086810); (CFCC 57393; GenBank ON114183, ON086815, ON086811).

HOST/DISTRIBUTION: on leaves of *Sonneratia apetala* in China.

NOTE: The four isolates of *Pestalotiopsis sonneratiae* clustered in a well-supported clade (ML/Bi = 93%/1) in FIG. 2 and appeared closely related to *P. etonensis*, *P. formosana*, *P. nanningensis*, *P. rhizophorae*, and *P. thailandica*. Morphologically, *P. sonneratiae* is similar to them in conidial size, but is found on a host in a different botanical family and order (TABLE 2; Ariyawansa & al. 2018, Crous & al. 2020, Li & al. 2021, Norphanphoun & al. 2019). Phylogenetically, *P. sonneratiae* can be distinguished by sequence data (nucleotide differences from *P. etonensis* in ITS: 3/505; tef1: 6/483; tub2: 11/447; from *P. formosana* in ITS: 2/499; tef1: 8/482; tub2: 9/445; from *P. nanningensis* in ITS: 8/505; tef1: 28/480; tub2: 26/442; from *P. rhizophorae* in ITS: 6/505; tef1: 6/476; tub2: 13/442; from *P. thailandica* in ITS: 5/506; tef1: 6/476; tub2: 12/442).

TABLE 2. Morphological comparison of *Pestalotiopsis sonneratae* and related species.

SPECIES	HOST	CONIDIAL SIZE (µm)	BASAL APPENDAGE LENGTH (µm)	NO. APICAL APPENDAGES	APICAL APPENDAGE LENGTH (µm)	REFERENCE
<i>P. etonensis</i>	<i>Sporobolus jacquemontii</i> (Poaceae)	15–21 × 4–7	2–4.5	3	6–16	Crous & al. 2020
<i>P. formosana</i>	— (Poaceae)	18–22 × 6–7	3–5	2	11–16	Ariyawansa & al. 2018
<i>P. nanningensis</i>	<i>Camellia oleifera</i> (Theaceae)	24–26.5 × 7–8	4.5–6.5	2–3	18–22.5	Li & al. 2021
<i>P. rhizophorae</i>	<i>Rhizophora apiculata</i> (Rhizophoraceae)	17.5–23 × 6–6.5	1.5–4.5	1–2	8–13	Norphanphoun & al. 2019
<i>P. sonneratae</i>	<i>Sonneratia apetala</i> (Lythraceae)	16–22 × 5.5–8.5	2.5–8	2–3	8–18	This study
<i>P. thailandica</i>	<i>Rhizophora apiculata</i> (Rhizophoraceae)	17.5–28 × 5.5–6.5	2.5–9.5	1–2	11–34	Norphanphoun & al. 2019

Discussion

In this study, disease samples with typical leaf spot symptom were observed and collected from *Sonneratia apetala* in Guangdong Province in China. Based on DNA sequence data of combined ITS, *tef1*, and *tub2* loci, *Pestalotiopsis sonneratae* is proposed here as the potential pathogen causing *S. apetala* leaf spot disease.

Another pestalotioid species *Neopestalotiopsis sonneratae* Norph. & al. [as “*sonneratae*”] was reported to be associated in Thailand with leaf spots on *Sonneratia alba* Sm. [Norphanphoun & al. 2019; as “*Sonneronata*”]. However, our *P. sonneratae* is obviously distinguished from *N. sonneratae* by pigmentation in the three median cells of the conidia (Norphanphoun & al. 2019).

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